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EXAMINER	
HILL, KEVIN KAI	
ART UNIT	**PAPER NUMBER**
1633	

DATE MAILED: 08/30/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/612,410	BENNETT, ROBERT P.	
	Examiner	Art Unit	
	Kevin K. Hill, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 June 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-156* is/are pending in the application.

4a) Of the above claim(s) *1-18,39-59,80-103,129-135 and 139-156* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *19-38,60-79,104-128 and 136-138* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date June 10, 2004 and Jan 24, 2006

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

Detailed Action

1. Applicant's election of the invention of Group II, claims 19-38, 60-79, 104-128 and 136-138 and the species of (b) one or more topoisomerase sites, the species (a) of Type I topoisomerase, the poxvirus topoisomerase species (a) vaccinia virus, the post-translational modification species (a) biotinylation, the amino acid sequence tag species (a) all or a portion of the *Klebsiella pneumoniae* oxalacetate decarboxylase, and the single nucleic acid structure species (b) linear in response to the Requirement for Restriction, filed on June 15, 2006 is acknowledged. Election was made without traverse.

2. Claims 1-18, 39-59, 80-103, 129-135, and 139-156 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

3. Claims 19-38, 60-79, 104-128 and 136-138 are under consideration.

Priority

4. Applicant's claim for priority under 35 U.S.C. 119(e) regarding the provisional application 60/393756, filed on July 8, 2002, the provisional application 60/396627, filed on July 19, 2002 and the provisional application 60/417172, filed on October 10, 2002, is acknowledged. According the effective priority date of the instant application is granted as July 8, 2002.

Information Disclosure Statement

5. Applicant has filed Information Disclosure Statements (IDSes) on July 3, 2003 and January 24, 2006, providing 96 references. The Examiner was able to consider these to the extent of time allowable and requests the Applicant to distinctly identify with a concise explanation of relevance any statements within a citation directly applicable to the instantly claimed invention.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

6. **Claims 19, 28, 30, 32, 34-38, 60, 69-71, 73, 75, 104, 113-116, 118-121 and 126-128 are** rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 45-55 of U.S. Patent No. 6,720,140. Although the conflicting claims are not identical, they are not patentably distinct from each other because the breadth of the instantly

claimed method(s) of producing a polynucleotide construct and subsequently defined terms is(are) encompassed with the patented method for *in vitro* cloning of a nucleic acid molecule.

With respect to the method(s) of cloning a nucleic acid molecule, Hartley et al (U.S. Patent No. 6,720,140) claim a method for *in vitro* cloning of a nucleic acid molecule comprising mixing a first vector comprising at least a first and second recombination sites, and a second vector comprising at least a third and fourth recombination sites, wherein said first and/or second vector further comprises a nucleic acid molecule to be cloned, and wherein said first and second recombination sites do not recombine with each other and said third and fourth recombination sites do not recombine with each other (see claim 45). The patented method steps encompass the method steps recited in Claims 19, 60 and 104 of the instant application, wherein a first nucleic acid molecule comprising a nucleotide sequence of interest flanked by at least a first and at least a second recombination sites that do not recombine with each other is contacted with a second nucleic acid molecule comprising at least a third and fourth recombination sites that do not recombine with each other under conditions favoring recombination between said first and third and between said second and fourth recombination sites, thereby producing a product polynucleotide construct. Although the patented method does not recite the third nucleic acid molecule and the second product polynucleotide construct, as recited in Claim 104 of the instant application, the open language using the term "comprising" does not limit the patented method to just the first and second nucleic acid molecule producing just the first product polynucleotide, as the patented method encompasses a second iteration of the recited method steps to produce a second product polynucleotide.

Claim 19 of the instant application recites the method step (c) "contacting said first nucleic acid molecule with said second nucleic acid molecule under conditions favoring recombination". The specification does not define "contacting", but one of ordinary skill in the art would recognize that the "conditions favoring recombination" would be in a reaction vessel or chamber, in a solution and in the presence of a recombinase. Hartley et al recite (claim 45) the method step (b) "incubating said mixture in the presence of at least one recombination protein under conditions sufficient to cause recombination". Thus, it would be obvious to one of ordinary skill in the art that, although phrased differently, the method step of "contacting...under

conditions favoring recombination" as recited in the instant application are encompassed by the "incubating said mixture in the presence of at least one recombination protein under conditions sufficient to cause recombination" method of Hartley et al.

Hartley et al use the generic term "vector" and "nucleic acid molecule" in their claim language. The Examiner has looked to the specification for definition(s) to better understand the nature of the invention. Hartley et al disclose that the invention relates to DNA, RNA, vectors and methods to effect exchange and/or to select for one or more desired products, and that the nucleic acid molecule may be chimeric and have the "desired characteristic(s) and/or DNA segment(s)" (column 4, lines 47-50; Abstract). Examples of desired DNA segments can be, but are not limited to, PCR products, large DNA segments, functional elements, genes or partial genes which encode useful nucleic acids or proteins (column 11, lines 49-53). Hartley et al generically define a "vector" as " a DNA that provides a useful biological or biochemical property to an Insert" (column 10, lines 62-65), wherein an "Insert Donor DNA molecule comprises a desired DNA segment flanked by a first recombination site and a second recombination site, wherein the first and second recombination sites do not recombine with each other" (column 4, lines 53-57). Thus, the breadth of the "vector" and "nucleic acid molecule" as recited in claim 45 of Hartley et al encompass the first, second and third nucleic acid molecules of the instant application as desired DNA molecule(s).

Because of the generic language used to define the metes and bounds of the invention, as described above, the Examiner has looked to working examples provided in the specification as embodiments of the invention that are necessarily embraced by the "vector" and "nucleic acid molecule" as recited in claim 45. Example 3 illustrates methods to subclone DNA fragments flanked by *attB* sites without stop codons to create fusion proteins (e.g. glutathione S-transferase, histidine tag) allowing one's protein of interest to be purified by affinity chromatography (column 22, lines 45-67). The specification of the instant application defines "amino acid sequence tag" as "amino acid sequences that are capable of being post-translationally modified, and/or amino acid sequences that are capable of being recognized by an antibody (or fragment thereof) or other specific binding reagents" (page 6, [0016]). The "affinity chromatography" disclosed by Hartley et al reads on "specific binding reagents" of the instant application. As such, it would be obvious to one of ordinary skill in the art that the product polynucleotide(s)

obtained by the method steps of claim 45 of Hartley et al, will yield a fusion protein(s) that comprises an amino acid sequence tag, e.g. glutathione S-transferase, histidine tag, fulfilling the definition(s) of the instant application. Similarly, it would be obvious to one of ordinary skill in the art that the product polynucleotide(s) obtained by the method steps of Claims 19, 60 and 104 of the instant application, will yield a fusion protein that was contemplated by Hartley et al.

Hartley et al recite the product polynucleotide is introduced into a host cell, and transformed cells containing the product polynucleotide are selected by the presence of a selectable marker, e.g. an antibiotic resistance gene or a toxic gene (see claims 53-55), method steps also recited in Claims 32, 73, 118-119 and 136-138 of the instant application. Hartley et al broadly defines "selectable marker" as a DNA segment that allows one to select for or against a molecule or a cell that contains it, often under particular conditions (column 9, lines 15-43). Furthermore, Hartley et al disclose that the first and/or second vector may comprise additional elements such as origins of replication, expression signals, selectable markers, DNA segments that can be used to isolate a desired molecule (e.g. specific protein binding sites) or toxic genes (column 5, lines 65-67, column 9, lines 15-43 and column 10, lines 7-30); embodiments disclosed in Claims 34, 75 and 120 of the instant application.

Thus, it would have been obvious to one of ordinary skill in the art that the method for *in vitro* cloning of a nucleic acid molecule and the nucleic acid molecule(s) as defined and claimed by Hartley et al are the same as the method for producing a polynucleotide construct and the nucleic acid molecule(s) of the instant application, as recited in Claims 19, 28, 30, 32, 34, 60, 69-71, 73, 75, 104, 113-116 and 118-120.

Hartley et al use the generic term "recombination protein" in their claim language, wherein a recombination protein may be Int, IHF, Xis, Hin, Gin, Cin, γδ, Cre, resolvase, or Flp (see claims 46-49), embodiments recited in Claims 37 and 127. The Examiner has looked to the specification for definition(s) to better understand the nature of the invention. Hartley et al broadly describes recombination proteins to include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites (column 9, lines 7-10). In particular, site-specific recombinases are defined as a type of recombinase which typically has at least the following four activities: (i)

recognition of one or two specific DNA sequences; (ii) cleavage of said DNA sequence or sequences, (iii) DNA topoisomerase activity involved in strand exchange, and (iv) DNA ligase activity to reseal the cleaved strands of DNA (column 10, lines 40-52). For example, Hartley et al discloses that members of the highly related resolvase family include $\gamma\delta$, Tn3, Hin, Gin and Cin (column 15, lines 26-30). Hartley et al do not recite the recombination protein embodiments Fis, TndX, XerC or XerD recited in the claims of the instant application, but do disclose Fis, Xis and IHF as recombination proteins (column 8, lines 63-65, see also columns 14-16). In regards to Hartley et al's functional definition, the specification of the instant application discloses that type 1B topoisomerases bind to and cleave a specific nucleotide sequence (page 38 [00117, line 6]), thus fulfilling parts (i) and (ii) of Hartley et al's definition of the claimed recombination protein. The art teaches that DNA type 1B topoisomerase also performs the remaining two Hartley et al defined functions, that is, (iii) DNA topoisomerase activity involved in strand exchange, and (iv) DNA ligase activity to reseal the cleaved strands of DNA (Shuman et al, AT16, page 324, section 4).

Thus, it would have been obvious to one of ordinary skill in the art that the recombination protein(s) as defined and claimed by Hartley et al are the same as the recombination and DNA topoisomerase proteins of the instant application, as recited in Claims 36-38, 60,76-79, 104, and 122-128.

Hartley et al use the generic term "recombination sites" in their claim language. The Examiner has looked to the specification for definition(s) to better understand the nature of the invention. Hartley et al define recognition sequences as particular DNA sequences which a protein, e.g. recombinase, recognizes and binds. For example, the recognition sequence for Cre recombinase is *loxP*... Other examples of recognition sequences are *attB*, *attP*, *attL* and *attR* sequences which are recognized by the Int recombinase enzyme (column 8, lines 47-67), embodiments that are recited in Claims 35 and 121 of the instant application. The specification of the instant application discloses that other suitable recombination sites include *psi*, *dif* and *cer* (page 34, [00110]), demonstrating functional equivalency of the claimed recombination sites for the method of producing a polynucleotide construct.

Thus, it would have been obvious to one of ordinary skill in the art that the recombination sites, as disclosed and claimed by Hartley et al are the same as the recombination sites as recited in Claims 35 and 121 of the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. **Claims 19, 35, 104 and 121 are rejected under 35 U.S.C. 112, first paragraph,** as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Vas-cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states that Applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." (See page 1117.) The specification does not clearly allow persons of ordinary skill in the art to recognize that (he or she) invented what is claimed." (See *Vas-cath* at page 1116).

Applicant is referred to the revised interim guidelines on written description published January 5, 2001 in the Federal Register, Volume 66, Number 5, page 1099-1111 (also available at www.uspto.gov).

The claimed invention is directed to cloning vectors utilizing a genus of recombination sites. Furthermore, Claims 35 and 121 recite "mutants, variants and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h) or (i) which retain the ability to undergo

recombination" The claims thus encompass potentially a very large genus of binding sites, with a vast number of permutations for each recited site.

In analyzing whether the written description requirement is met for genus claims, it is first determined whether a representative number of species have been described by their complete structure. In the instant case, *attB* 1-10, *attP* 1-10, *attL* 1-10, *attR* 1-10, *loxP* and *loxP*511, and the canonical *psi*, *dif*, *cer* and *frt* recombination sites are the only species whose complete structure is disclosed.

Next, then, it is determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics (i.e. other than nucleotide sequence), specific features and functional attributes that would distinguish different members of the claimed genus. In the instant case, the only other identifying characteristic is that the recombination sites would be recognized by a recombinase enzyme and will not cross-react with recombination sites of other mutant types and retain the ability to undergo recombination (page 4, [0012]; page 47, [00139]).

However, there is not sufficient information set forth in the specification or available in the prior art to permit a determination of which mutations to each binding site will retain activity and which will not. Furthermore, it is not apparent that the art teaches any theory or algorithm which would permit the determination of whether a particular sequence holds an active recombination site as claimed. It is noted that all these recombination sites vary greatly in structure and function and therefore each represents a subgenus. Again, the members of any of the subgenuses themselves would have very different structure and the specification does not provide any description of any identifying characteristics of the species of the subgenuses. As such, there is an insufficient structure-function relationship between the sequence and the function or activity as a recombinase binding site.

Accordingly, this limited information is not deemed sufficient to reasonably convey to one skilled in the art that the applicant is in possession of the broad genus of *attB*, *attP*, *attL*, *attR*, *loxP*, *psi*, *dif*, *cer* and *frt* recombination sites and "mutants, variants and derivatives" thereof, besides *attB* 1-10, *attP* 1-10, *attL* 1-10, *attR* 1-10, *loxP* and *loxP*511, and the canonical *psi*, *dif*, *cer* and *frt* recombination sites, at the time the application was filed. Thus it is concluded that the written description requirement is not satisfied for the claimed genus.

8. **Claims 19-38, 60-79, 104-128 and 136-138 are rejected under 35 U.S.C. 112, first paragraph**, because the specification, while being enabling for the *attB* 1-10, *attP* 1-10, *attL* 1-10, *attR* 1-10, *loxP* and *loxP*511, and the canonical *psi*, *dif*, *cer* and *frt* recombination sites does not reasonably provide enablement for all "mutants, variants and derivatives" of said recombination sites, as recited in Claims 35 and 121. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

Although Claims 60-79 are drawn to topoisomerase sites and do not explicitly recite the use of recombinase sites, the claims use the term "comprising" which is recognized as open language, and thus encompass the possible presence and use of recombinase sites. Furthermore, given the instant disclosure and the language of Claims 104-128, Applicant has clearly contemplated the combined use of recombinase sites with topoisomerase sites to rapidly clone a polynucleotide sequence of interest.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention. If not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2ds 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification. Therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention. And thus, skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The Nature of the Invention and The Breadth of the Claims

The inventive concept in the instant application is to use recombinase proteins that recognize specific nucleotide sequences, recombination sites, to facilitate the rapid cloning and subcloning of one's polynucleotide of interest. The claimed invention(s) is directed to recombinational cloning vectors utilizing a broad genus of recombination sites. Furthermore, Claims 35 and 121 recite "mutants, variants and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h) or (i) which retain the ability to undergo recombination" The claims thus encompass potentially a very large genus of binding sites, with a vast number of permutations for each recited site.

The State of the Prior Art, The Level of One of Ordinary Skill and The Level of Predictability in the Art

However, there is not sufficient information available in the prior art to permit a determination of which mutations to each binding site will retain activity and which will not. Furthermore, it is not apparent that the art teaches any theory or algorithm which would permit the determination of whether a particular sequence holds an active recombination site as claimed. As such, there is an insufficient structure-function relationship between the sequence and the function or activity as a recombinase binding site.

Campbell et al (October 30, Gene 300: 13-18, 2002) discusses specificity in DNA recognition by phage integrases. In particular, Campbell teaches that DNA-protein recognition is necessary for two domains of the integrase (Int) protein: the C-terminal domain recognizes the core sites immediately flanking the exchange point on the DNA (*att* site); whereas the N-terminal domain recognizes "arm sites" that are located in flanking DNA further removed, by as much as 240bp from the exchange point (page 14, column 1). Similarly, Santoro and Schultz (April 2, Proc. Natl. Acad. Sci. USA 99(7): 4185-4190, 2002) teach that each subunit of Cre binds to a *loxP* half-site through a multitude of protein-DNA contacts, the majority of DNA interactions appear to be nonspecific, involving the DNA backbone (page 4187, column 2). Direct interactions between the Cre recombinase and the DNA bases, although relatively few in number, are critical for Cre function, as the enzyme must exhibit high sequence specificity in

binding to its recombination site, wherein the sequence of base pairs 2-7 of the *loxP* half-sites appears to be particularly important for Cre binding. Santoro and Schultz teach a strategy to identify variant Cre recombinases that would recognize novel *loxP* sites, but not the wildtype loxP site and found that recombinases with relaxed specificity towards wildtype and variant recognition sites are relatively more abundant than recombinases with specific recognition to a variant site. Thus, there is significant unpredictability in the requisite mutations for a modified recombinase to selectively recognize a first variant recognition site and that would not recognize or recombine with a second non-variant recombination site.

The Amount of Direction Provided by the Inventor

The specification teaches that the recombination sites may be any nucleic acid sequence that can serve as a substrate in a recombination reaction, and may be wild-type or naturally occurring sites, or modified or mutant sites (page 33, [00109]). However, the specification does not teach several important considerations. For instance, the specification does not provide specific guidance to one of ordinary skill in the art to determine which mutations to each of the disclosed recombinase binding sites will retain activity and which will not.

The Quantity of Any Necessary Experimentation to Make or Use the Invention

Thus, the quantity of necessary experimentation to make or use the invention as claimed, based upon what is known in the art and what has been disclosed in the specification, will create an undue burden for a person of ordinary skill in the art to demonstrate which "mutants, variants and derivatives of the recombination sites...retain the ability to undergo recombination".

In conclusion, the specification fails to provide any guidance as to how an artisan would have dealt with the art-recognized limitations of the claimed method commensurate with the scope of the claimed invention and therefore, limiting the claimed invention(s) to the *attB* 1-10, *attP* 1-10, *attL* 1-10, *attR* 1-10, *loxP* and *loxP*511, and the canonical *psi*, *dif*, *cer* and *frt* recombination sites, is proper.

9. **Claims 19, 35, 60-79 and 104-128 are rejected under 35 U.S.C. 112, second paragraph**, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

With respect to Claims 60 and 104, and thus all claims ultimately depending therefrom, the claims are drawn to a "nucleic acid molecule comprising... at least one topoisomerase." However, a nucleic acid cannot comprise an enzyme, which is a protein. Rather, it might encode a protein, in this instance, the topoisomerase.

With respect to Claims 35 and 121 recite "mutants, variants and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h) or (i) which retain the ability to undergo recombination." However, it is not clear what a mutant, variant or derivative encompasses. For example, if a recited *lox* site were completely mutated into a site for another recombinase, in particular one not recited in Claims 35 and 121, would the newly mutated site still be encompassed within Claims 35 and 121? This is not clear, and as such, the metes and bounds of the instant claim are indefinite.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

10. **Claims 19, 28, 30-32 and 34-37 are rejected under 35 U.S.C. 102(b)** as being anticipated by Hartley et al (AT8).

The claims are drawn to a method of producing a polynucleotide construct that encodes a fusion protein that comprises an amino acid sequence tag, wherein a first nucleic acid molecule inserts into a second nucleic acid molecule by means of a recombinase.

With respect to the limitations of Claim 19, Hartley et al teach methods of cloning one's gene of interest into expression vectors using recombinases, resulting in fusion proteins, wherein the amino acid sequence tag is a histidine tag, a GST-fusion or a thioredoxin-fusion protein (page 1790, Table 1). Figure 1 (page 1789) illustrates variations of recombinational cloning methods, including a first nucleic acid molecule that comprises a nucleotide sequence of interest flanked by at least a first and at least a second recombination sites that do not recombine with each other and a second nucleic acid molecule that comprises at least a third and fourth recombination sites that do not recombine with each other, as recited in Claim 19.

With respect to the limitations of Claim 28, Hartley et al teach numerous "Destination Vectors" (page 1790, Table 1).

With respect to the limitations of Claims 30-32, Hartley et al teach that the first nucleic acid molecule may be linear, as exemplified by a PCR product and the bacterial transformation of the product polynucleotide (page 1790, columns 1 and 2).

With respect to the limitations of Claim 34, Hartley et al teach that the second nucleic acid molecule comprises instantly recited elements, such as a selectable marker, a cloning site, a restriction site, a promoter, an origin of replication, a gene product which allows for negative selection, and a gene or partial gene (page 385, Figure 2).

With respect to the limitations of Claim 35-37, Hartley et al teach the recombinases Int, IHF and Xis, and the recombination sites *attL*, *attR*, *attB* and *attP* (page 1789, see also Figure 1). Thus, 19, 28, 30-32 and 34-37 are anticipated by Hartley et al.

11. **Claims 60, 69-70, 72-73 and 75-79 are rejected under 35 U.S.C. 102(b)** as being anticipated by Heyman et al (Genome Research 9: 383-392, 1999).

The claims are drawn to a method of producing a polynucleotide construct that encodes a fusion protein that comprises an amino acid sequence tag, wherein a first nucleic acid molecule inserts into a second nucleic acid molecule by means of topoisomerase.

With respect to the limitations of Claim 60, Heyman et al teach methods of cloning one's gene of interest into expression vectors using topoisomerases, resulting in fusion proteins, wherein the amino acid sequence tag is a V5 epitope and/or a histidine tag (page 385, Figure 2). Figure 1 (page 384) illustrates variations of topoisomerase cloning methods, including a second nucleic acid molecule that comprises at least two topoisomerase recognition sites, as recited in Claim 60.

With respect to the limitations of Claim 69, Heyman et al teach two vectors (page 385, Figure 2).

With respect to the limitations of Claims 70 and 72, Heyman et al teach that the first nucleic acid molecule may be linear, as exemplified by a PCR product (page 384, Figure 1).

With respect to the limitations of Claim 73, Heyman et al teach the bacterial transformation of the product polynucleotide (page 385, Phase III).

With respect to the limitations of Claim 75, Heyman et al teach that the second nucleic acid molecule comprises instantly recited elements, such as a selectable marker, a cloning site, a restriction site, a promoter, an origin of replication, and a gene or partial gene (page 385, Figure 2).

With respect to the limitations of Claim 76-79, Heyman et al teach that the topoisomerase is the vaccinia virus-encoded eukaryotic type I topoisomerase (page 383, Introduction, column 1).

Thus, Claims 60, 69-70, 72-73 and 75-79 are anticipated by Heyman et al.

12. **Claims 19, 28, 30-32, 34-38, 60, 69-73, 75-79, 104 and 113-128 are rejected under 35 U.S.C. 102(e)** as being anticipated by Hartley et al (U.S. Patent No. 6,277,608 B1).

The applied reference has a common assignee with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

With respect to the limitations of Claims 19, 60, 104 and 126, Hartley et al teach/claim a method for cloning or subcloning desired nucleic acid molecules comprising (A) (i) combining one or more "Insert Donor" molecules comprising one or more nucleic acid segments flanked by two or more recombination sites, wherein said recombination sites do not substantially recombine with each other; (ii) two or more different "Vector Donor" molecules, each comprising two or more recombination sites, wherein said recombination sites do not substantially recombine with each other; and (iii) one or more site specific recombination proteins, and (B) incubating the combination under conditions sufficient to transfer one or more of said nucleic acid segments into said different Vector Donor molecules, thereby producing two or more different product molecules (see claim 1). The patented method steps encompass the method steps recited in Claims 19, 60 and 104 of the instant application, wherein a first nucleic acid molecule comprising a nucleotide sequence of interest flanked by at least a first and at least a second recombination sites that do not recombine with each other is contacted with a second nucleic acid molecule comprising at least a third and fourth recombination sites that do not recombine with each other under conditions favoring recombination between said first and third and between said second and fourth recombination sites, thereby producing a product polynucleotide construct. Although the recited methods of Claims 19 and 60 do not recite two or more different vector donor molecules, as claimed by Hartley et al, the open language using the term "comprising" does not limit the recited methods to just the first and second nucleic acid molecule producing just the first product polynucleotide, as the methods encompass a second iteration of the recited method steps and/or additional vector nucleic acid molecules in the reaction to produce the desired product polynucleotide.

With respect to the limitations of Claims 28, 30-31, 69-72 and 113-117, Hartley et al teach/recite that the Insert Donor molecule may be genomic DNA, cDNA, or produced by chemical synthesis (see claims 2-4); physical and chemical structural features structurally indistinguishable from the first nucleic acid molecules disclosed in the instant application. Furthermore, the vector molecules may either be linear or circular (column 17, line 40), and thus structurally indistinguishable from the second and third nucleic acid molecules recited in the instant application.

With respect to the limitations of 32, 73 and 118-119, Hartley et al discloses the host cell to be "any prokaryotic or eukaryotic organism that can be a recipient of the recombinational cloning product" (column 13, lines 50-54, see also column 6, lines 27-47). Furthermore, Hartley et al successfully demonstrates the method of cloning a polynucleotide molecule and transforming *E. coli* host cells with the product polynucleotide (column 32, Example 1).

With respect to the limitations of 34, 75 and 120, Hartley et al teach the vector nucleic acid molecules may contain one or more selectable markers (columns 15-16) which describe the selectable marker, nucleotide sequence encoding a gene product which allows for negative selection, DNA segments that encode products which suppress the activity of a gene product (a gene or partial gene), DNA segments that otherwise inhibit the activity of any of the previously described DNA segments (operator/operon/repressor), DNA segments that can be used to isolate or identify a desired molecule (cloning site, restriction site, promote, operator, operon, origin of replication, etc….) (column 15, lines 1-30).

With respect to the limitations of 35 and 121, Hartley et al claim/disclose that at least one of the recombination sites is an *att* or *loxP* site (see claim 21). Hartley et al define recognition sequences as particular DNA sequences which a protein, e.g. recombinase, recognizes and binds. For example, the recognition sequence for Cre recombinase is *loxP*… Other examples of recognition sequences are *attB*, *attP*, *attL* and *attR* sequences which are recognized by the Int recombinase enzyme (column 14, lines 28-45), embodiments that are recited in Claims 35 and 121 of the instant application. The specification of the instant application discloses that other suitable recombination sites include *psi*, *dif* and *cer* (page 34, [00110]), demonstrating functional equivalency of the claimed recombination sites for the method of producing a polynucleotide construct.

With respect to the limitations of 36-38/60, 76-79/104 and 122-128, Hartley et al claim that at least one of the recombination proteins is Int, Cre, Flp or Res (see claim 23). Hartley et al broadly describes recombination proteins to include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites (column 14, lines 55-60). Furthermore, site-specific recombinases are defined as a type of recombinase which typically has at least the following four activities: (i) recognition of one or two specific DNA sequences; (ii) cleavage of said DNA sequence or

sequences, (iii) DNA topoisomerase activity involved in strand exchange, and (iv) DNA ligase activity to reseal the cleaved strands of DNA (column 16, lines 45-51). For example, Hartley et al discloses that members of the highly related resolvase family include γδ, Tn3, Hin, Gin and Cin (column 24, lines 5-7). Hartley et al do not recite the recombination protein embodiments Fis, TndX, XerC or XerD recited in the instant application, but do disclose Fis, Xis and IHF as recombination proteins (column 14, lines 45-47). In addition to the shared claimed recombination proteins, the specification of the instant application discloses that type 1B topoisomerases bind to and cleave a specific nucleotide sequence (page 38 [00117, line 6]), thus fulfilling parts (i) and (ii) of Hartley et al's definition of the claimed recombination protein. The art teaches that DNA type 1B topoisomerase also performs the remaining two Hartley et al defined functions, that is, (iii) DNA topoisomerase activity involved in strand exchange, and (iv) DNA ligase activity to reseal the cleaved strands of DNA (Shuman et al, AT16, page 324, section 4). Furthermore, Hartley et al teaches that topoisomerases may be used to introduce recombination sites in accordance with the invention (column 8, lines 20-25).

With respect to the product polynucleotide construct containing a fusion protein that comprises an amino acid sequence tag, Hartley et al disclose that the invention relates to DNA, RNA, vectors and methods to effect exchange and/or to select for one or more desired products, and that the nucleic acid molecule may be chimeric and have the "desired characteristic(s) and/or DNA segment(s)" (Abstract). Examples of desired DNA segments can be, but are not limited to, PCR products, large DNA segments, functional elements, genes or partial genes which encode useful nucleic acids or proteins (column 20, lines 13-20), as exemplified by Example 3 to create fusion proteins (e.g. glutathione S-transferase, histidine tag) (column 35). The specification of the instant application defines "amino acid sequence tag" as "amino acid sequences that are capable of being post-translationally modified, and/or amino acid sequences that are capable of being recognized by an antibody (or fragment thereof) or other specific binding reagents" (page 6, [0016]). As such, the product polynucleotide(s) obtained by the method steps of Claims 19, 60 and 104 will yield a fusion protein that comprises an amino acid sequence tag, e.g. glutathione S-transferase or histidine tag, which fulfill the definition of the instant application and are contemplated products of the patented method of Hartley et al.

Thus, Claims 19, 28, 30-32, 34-38, 60, 69-73, 75-79, 104 and 113-128 are anticipated by Hartley et al.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. **Claims 19-27, 33, 60-68, 74 and 104-112 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Hartley et al (U.S. Patent No. 6,277,608 B1) as applied to the method of producing a polynucleotide construct that encodes a fusion protein, and in further view of Hearn and Acosta (J. Molecular Recognition 14: 323-369, 2001), as applied to the limitations of fusion proteins comprising amino acid sequence tags and amino acid protease cleavage sites, as evidenced by Cronan et al (AR5) and Arienne et al (AR1), as applied respectively to the limitations of biotinylation and enterokinase protease.

The claims are drawn to a method of producing a polynucleotide construct that encodes a fusion protein that comprises an amino acid sequence tag—the preferred embodiment being biotinylation and an amino acid sequence that is capable of being cleaved by one or more proteases—the preferred embodiment being enterokinase.

Hartley et al teach a method for cloning or subcloning desired nucleic acid molecules using recombination proteins that both teach and encompass the recombination proteins recited in the instant application, wherein the invention relates to nucleic acid molecules that may be chimeric and have the "desired characteristic(s) and/or DNA segment(s)" (Abstract). Examples of desired DNA segments can be, but are not limited to, functional elements, genes or partial genes which encode useful nucleic acids or proteins (column 20, lines 13-20), as exemplified by

Example 3 to create fusion proteins (e.g. glutathione S-transferase, histidine tag) (column 35). The specification of the instant application defines "amino acid sequence tag" as "amino acid sequences that are capable of being post-translationally modified, and/or amino acid sequences that are capable of being recognized by an antibody (or fragment thereof) or other specific binding reagents" (page 6, [0016]). As such, the product polynucleotide(s) obtained by the method steps of Hartley et al will yield a fusion protein that comprises an amino acid sequence tag, e.g. glutathione S-transferase or histidine tag, which fulfill the definition of the instant application.

Hartley et al do not explicitly teach the amino acid sequence tag be capable of post-translational modification, specifically biotinylation, nor that the polynucleotide construct also encode an amino acid sequence capable of being cleaved by a protease, specifically enterokinase; however, at the time of the invention, Hearn and Acosta reviewed the history of peptide fusion handles and affinity cassette methods, alone or in combination with amino acid sequences capable of being cleaved by a protease, used in the art to produce and purify recombinant proteins, summarizing the general knowledge and skill in the art for over twenty years. "In all cases, the strategy [of attaching 'affinity tags' or 'peptide handles'] seeks to achieve the most optimal affinity interactions and separation productivity at the laboratory and process scale level" (page 323, column 2). The appropriate and skillful selection of these affinate-affinant systems for a particular family of recombinant proteins forms the basis of the "molecular cassette concept" of high-resolution affinity chromatographic separation of proteins (page 324, column 2). Thus, the art recognizes the modular nature of 'affinity tags' or 'peptide handles' and the routine and common practice to substitute or modify one amino acid tag with another amino acid tag, and even combine multiple amino acid sequence tags, as per the nature and desire of the artisan. Hearn and Acosta teach representative peptide tags, including among others, biotinylation tags (page 334, Table 3). Hearn and Acosta also summarized the use of endopeptidases, including among others, enterokinase (page 332, Table 1), commonly used in the art at the time of filing of the instant application to cleave one's protein of interest from the affinity tag (page 334, Table 3) and facilitate protein purification. For example, Hearn and Acosta reference the PinPoint™ system (Promega, USA), in which the product polynucleotide encodes a fusion protein that

contains an amino acid sequence tag capable of biotinylation, in combination with an endoprotease cleavage site, e.g. Factor Xa (page 351, columns 1-2, joining paragraph).

Hearn and Acosta teach that the number of different tag systems of various molecular sizes, with different binding interactions and affinities, can be selected or designed for fusion to virtually any target protein that can be cloned and expressed in a microbial or eukaryotic host (page 336, column 1, section 5). In particular, Hearn and Acosta make reference to Cronan et al, who taught that there are "*obvious advantages*" (*emphasis added*) of fusing a protein of interest to a protein segment recognized by a coenzyme ligase, such as (i) the fusion proteins could be specifically labeled by growth of cell cultures in the presence of labeled coenzyme; (ii) if the coenzyme possessed specific binding toward an immobilized ligand, the fusion protein could be readily purified, perhaps in a native form, and (iii) the protein segment fused to the protein of interest would be much smaller than those commonly used, thus giving less alteration of the chemical and biological properties of the protein of interest. Biotin has distinct advantages over the other coenzyme modification groups, the main advantage being the existence of two proteins, avidin and streptavidin, that strongly and specifically bind biotin (Introduction, columns 1-2, joining paragraph). Cronan et al teach the creation of fusion proteins, exemplified using the biotin protein segments derived from the *P. shermanii* transcarboxylase 1.3S subunit, the *E. coli* acetyl CoA carboxylase BCCP subunit, a protein sequence encoded by a cDNA from tomato, and the *K. pneumoniae* ODC alpha subunit for biotinylation, as recited in Claims 25-26, 66-67 and 110-111.

It would have been obvious to one of ordinary skill in the art to modify the polynucleotide construct of Hartley et al that yields a fusion protein with an amino acid tag capable of biotinylation as taught in the instant application with a reasonable chance of success because Hearn and Acosta teach that, for over twenty years, it has been common practice to those of ordinary skill in the art to design and skillfully select peptide fusion handles and affinity cassette methods. Furthermore, Cronan et al successfully demonstrate the creation of fusion proteins capable of becoming biotinylated using protein segments derived from the *P. shermanii* transcarboxylase 1.3S subunit, the *E. coli* acetyl CoA carboxylase BCCP subunit, and the *K. pneumoniae* ODC alpha subunit. An artisan would be motivated to use an amino acid sequence tag capable of biotinylation because Cronan et al teach that the extremely specific and high

affinity binding of biotin by avidin and streptavidin results in specific detection systems of very high sensitivity, which may also be used to purify the biotinylated proteins (page 10330, columns 1-2, joining paragraph) and it has been common practice to those of ordinary skill in the art to design and skillfully select peptide fusion handles and affinity cassette methods to achieve optimal production and purification of recombinant proteins. Thus, epitope-tagging heterologous proteins via post-translational biotinylation is a well-recognized approach in the art.

It also would have been obvious to one of ordinary skill in the art to modify the polynucleotide construct of Hartley et al that yields a fusion protein to also comprise an amino acid sequence for protease cleavage as taught in the instant application with a reasonable chance of success because Hearn and Acosta had summarized the common practice in the art regarding the successful use of 'affinity tags' in combination with amino acid sequences capable of being cleaved by a protease, e.g. enterokinase, as recited in the instant application. Furthermore, Airenne et al demonstrated the successful method of peptide tagging (e.g. avidin) a polypeptide of interest (e.g. Hevein) with a proteolytic cleavage site for Factor Xa, enterokinase, thrombin and tobacco etch virus to cleave Hevein from the amino acid tag and facilitate isolation and purification of the Hevein polypeptide (page 143, c1), thus demonstrating the art-recognized functional equivalence of the utilizing any of these four proteases to cleave one's protein of interest from the amino acid sequence tag. An artisan would be motivated to include an amino acid sequence capable of being cleaved by a protease when creating a fusion protein comprising one's protein of interest and a biotinylated amino acid sequence tag because Cronan et al teaches that the extremely tight binding of biotin and biotinylated proteins to avidin or streptavidin cannot be reversed by elution with biotin, and thus elution of bound proteins requires much harsher methods (page 10330, columns 1-2, joining paragraph). Cronan et al suggest that one approach to address this challenge is to engineer a site for protease cleavage (e.g. the PinPoint™ system, Promega, USA). Such a fusion protein could be bound to immobilized avidin or streptavidin, washed free of contaminating proteins, and simultaneously eluted in native form (and freed of the biotinylated segment) by treatment of the column matrix with the designated protease. Avidin and streptavidin are extremely insensitive to proteases in general, and since the amino acid sequences of both proteins are known, selection of proteases unable to cleave avidin

and streptavidin is readily accomplished. Furthermore, Airenne et al teach that protease cleavage sites can easily be attached to the fusion protein by adding the appropriate sequences for cloning.

Thus, the invention(s) is(are) *prima facie* obvious.

14. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kevin K. Hill, Ph.D. whose telephone number is 571-272-8036. The examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave T. Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.
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